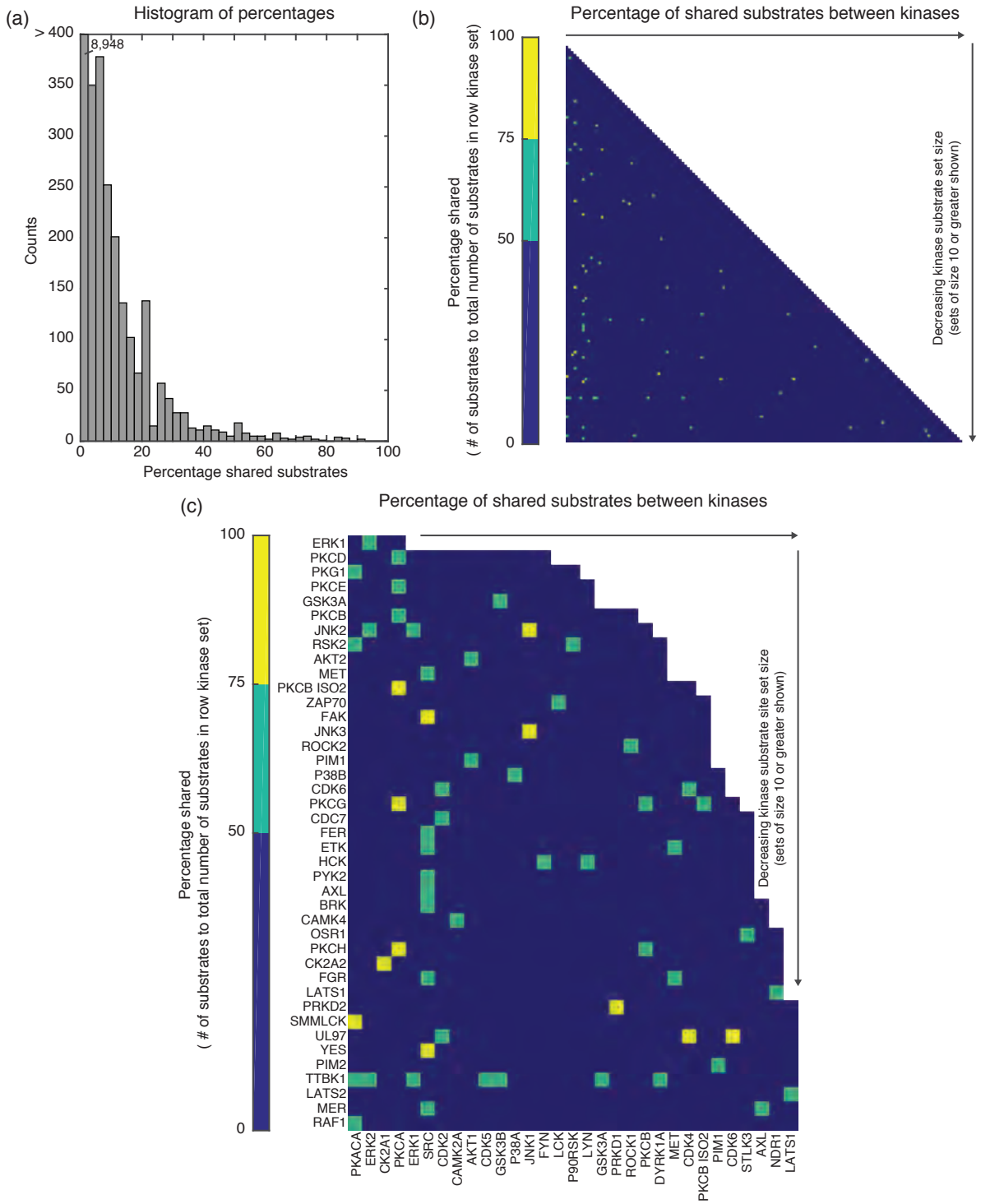

Figure S1. Overlap between kinase-substrate sets. (a) Histogram of percentages of shared substrates between kinase-substrate sets. (b) Heatmap detailing percentage of substrate sites shared between kinase-substrate sets of size 10 or greater. Numerical data in presented in Table S1. (c) Kinase-substrate sets from panel (b) that share at least 50% of substrates with at least one other set.

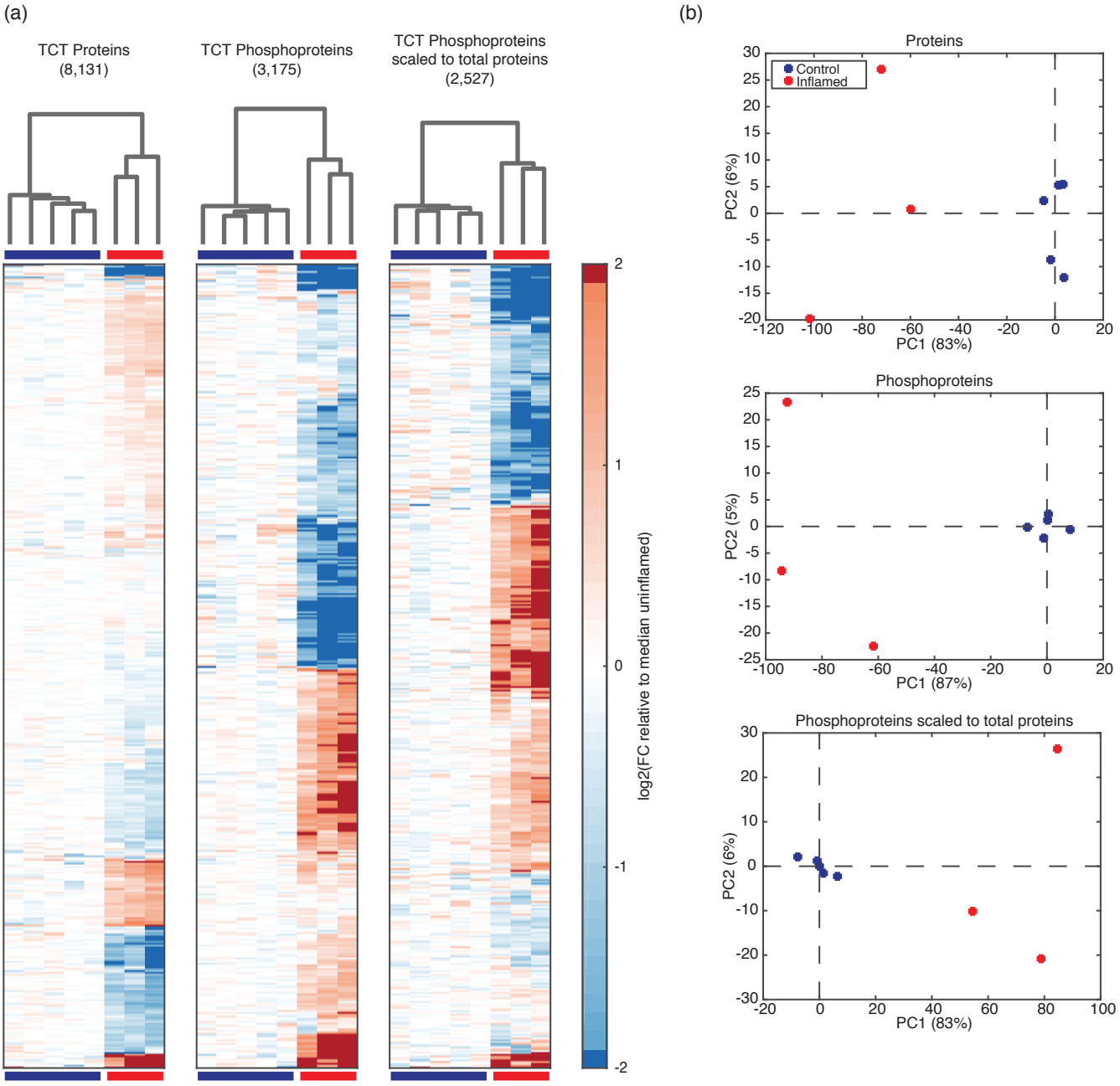

Figure S2. Distinct phosphoproteomic changes in the TCT model. (a) Unsupervised hierarchical clustering analysis using Euclidean distance metric and average linkage method of proteomic, phosphoproteomic, and phosphoproteomic scaled to total proteomic data from the TCT model. Fold change (FC) is taken with respect to the median value of the control samples. (b) Principal component analysis (PCA) was conducted on data transformed as log2(FC relative to median control). Percentages indicate fraction of variance captured by respective principal component (PC) axis. Data are from the analysis of 5 control (non-inflamed) colons and 3 inflamed colons.

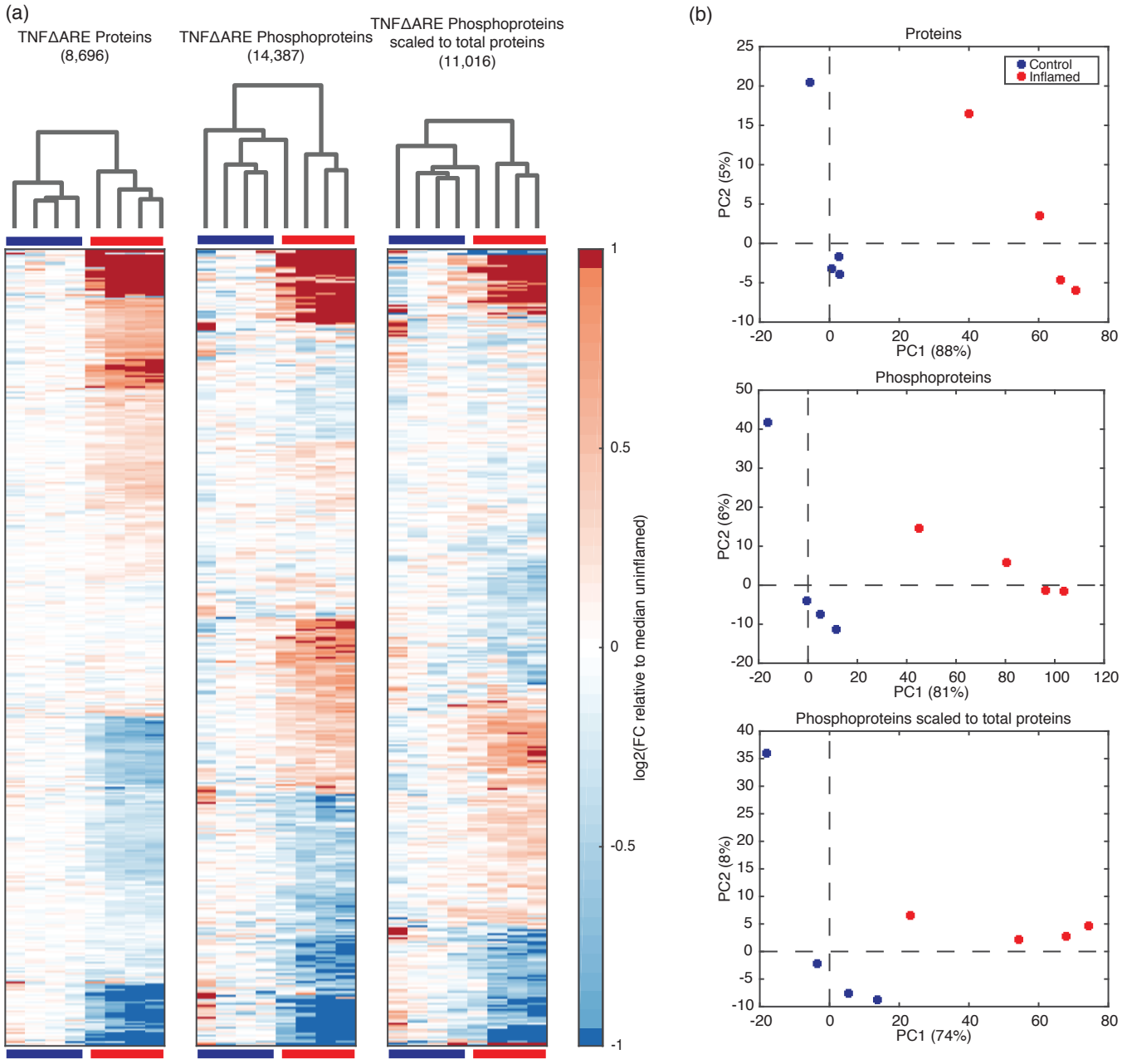

Figure S3. Distinct phosphoproteomic changes in the TNFΔARE model. (a) Unsupervised hierarchical clustering analysis using Euclidean distance metric and average linkage method of proteomic, phosphoproteomic, and phosphoproteomic scaled to total proteomic data from the TNFΔARE model. Fold change (FC) is taken with respect to the median value of the control samples. (b) Principal component analysis was conducted on data transformed as log2(FC relative to median control). Percentages indicate fraction of variance captured by respective principal component (PC) axis. Data are from the analysis of 4 control (non-inflamed) small intestines and 4 inflamed small intestines.

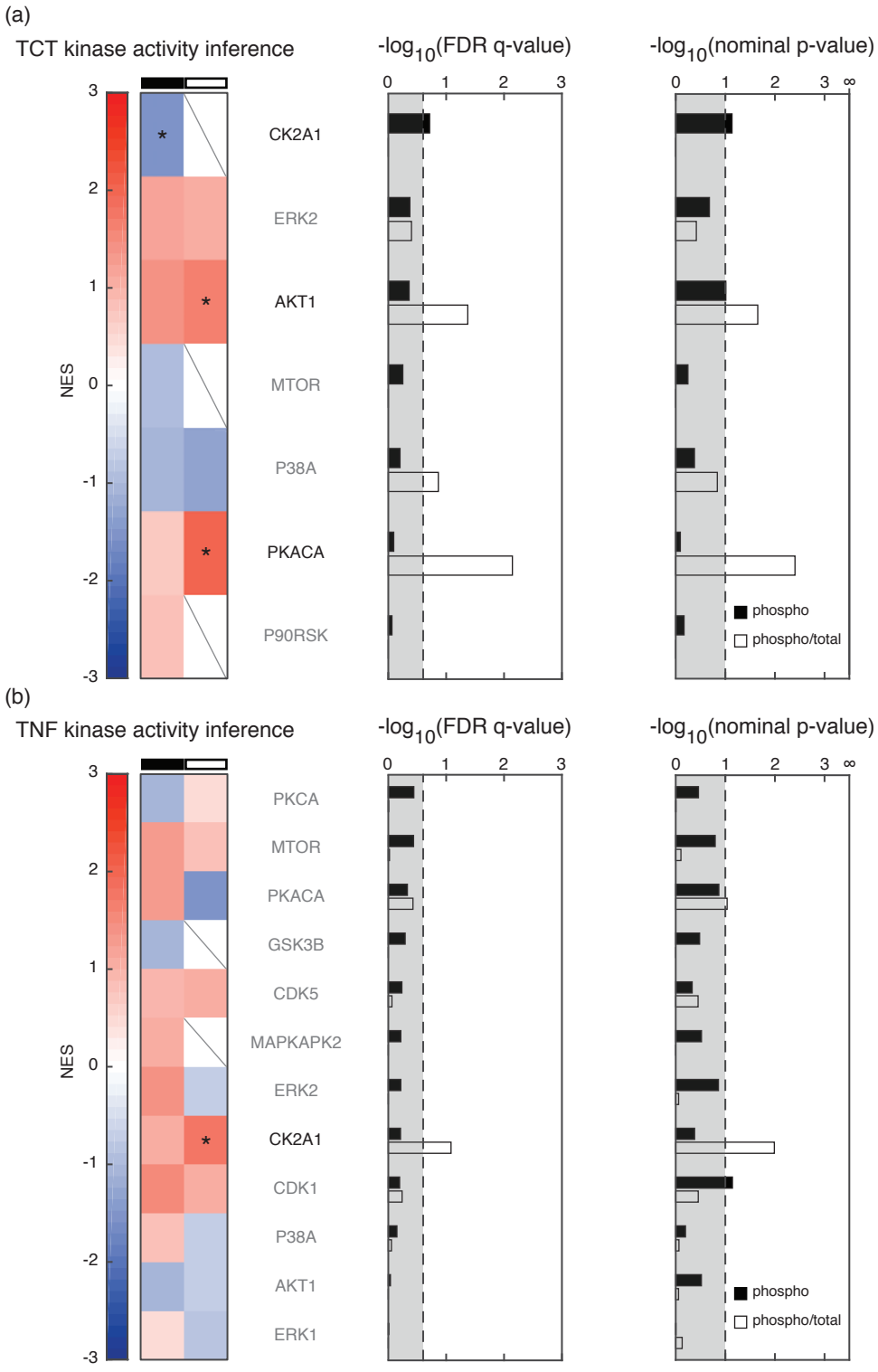

Figure S4. SKAI without expansion. SKAI conducted on phosphoproteomic data (phospho, black) and phosphoproteomic scaled to total proteomic data (phospho/total, white) from (b) TCT and (c) TNFΔARE models using the unexpanded kinase-substrate sets for mouse. Enrichment compares the inflamed to control samples. Each line in the heat map represents the normalized enrichment score (NES) associated with a kinase (set size ≥ 5). Slashes indicate the kinase's respective substrate set was not detected in the analysis due to insufficient (< 5) substrates in the respective dataset. Bar graphs present FDR q-values and nominal p-values for each kinase; thresholds are at an FDR q-value < 0.25 and nominal p-value < 0.1. Kinases shown in bold and marked with an * have results that meet these thresholds.

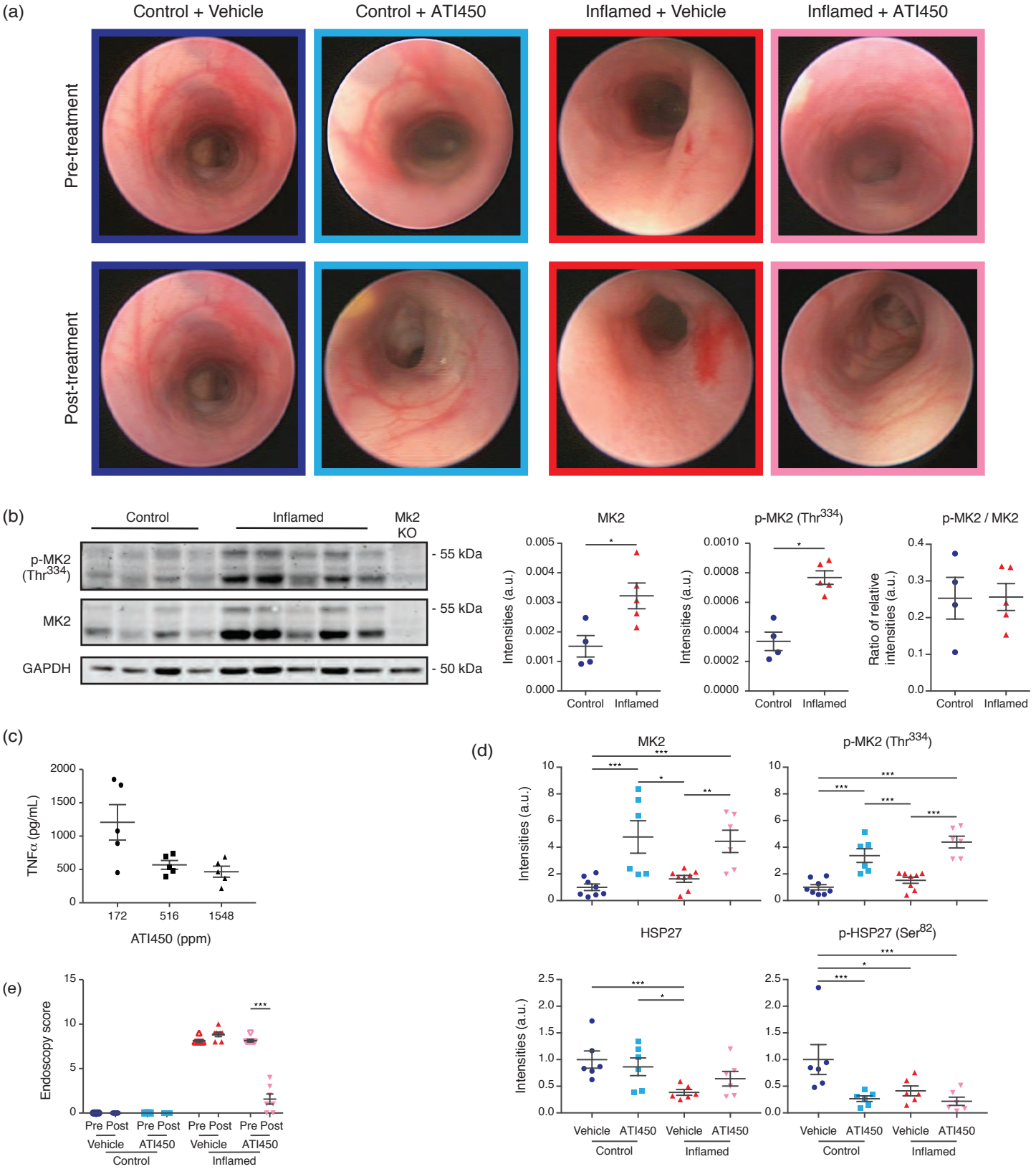

Figure S5. ATI450 treatment alleviates inflammation. (a) Representative mouse colonoscopy of vehicle- and ATI450-treated mice pre-treatment and post 2-week continuous treatment. Images are representative of 7 mice per treatment group. (b) Immunoblotting for MK2 and p-MK2 (Thr334) in small intestinal protein lysates from animals with and without ileitis (TNFΔARE model system). Lysates from MK2 knockout (KO) mice were used as a negative control. Graphs represent quantification of bands from the western blot, 4-5 mice/group. (c) ATI450 dosing studies of MK2 pathway inhibition. LPS-induced TNFα secretion as a measure of MK2 pathway inhibition by ATI450. Data from a single experiment with 5 mice per group. (d) Immunoblot quantification of MK2, p-MK2 (Thr334), HSP27, and p-HSP27 (Ser82), in lysates from colon tissue of ATI450-treated animals. Graphs represent quantification of western blots from 6-8 mice/group. (e) Endoscopy scores for animals pre- and post-treatment in all four experimental groups, with 7 mice / group (note statistical tests only compare pre-post). Error bars shown as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, by Wilcoxon Rank Sum test.

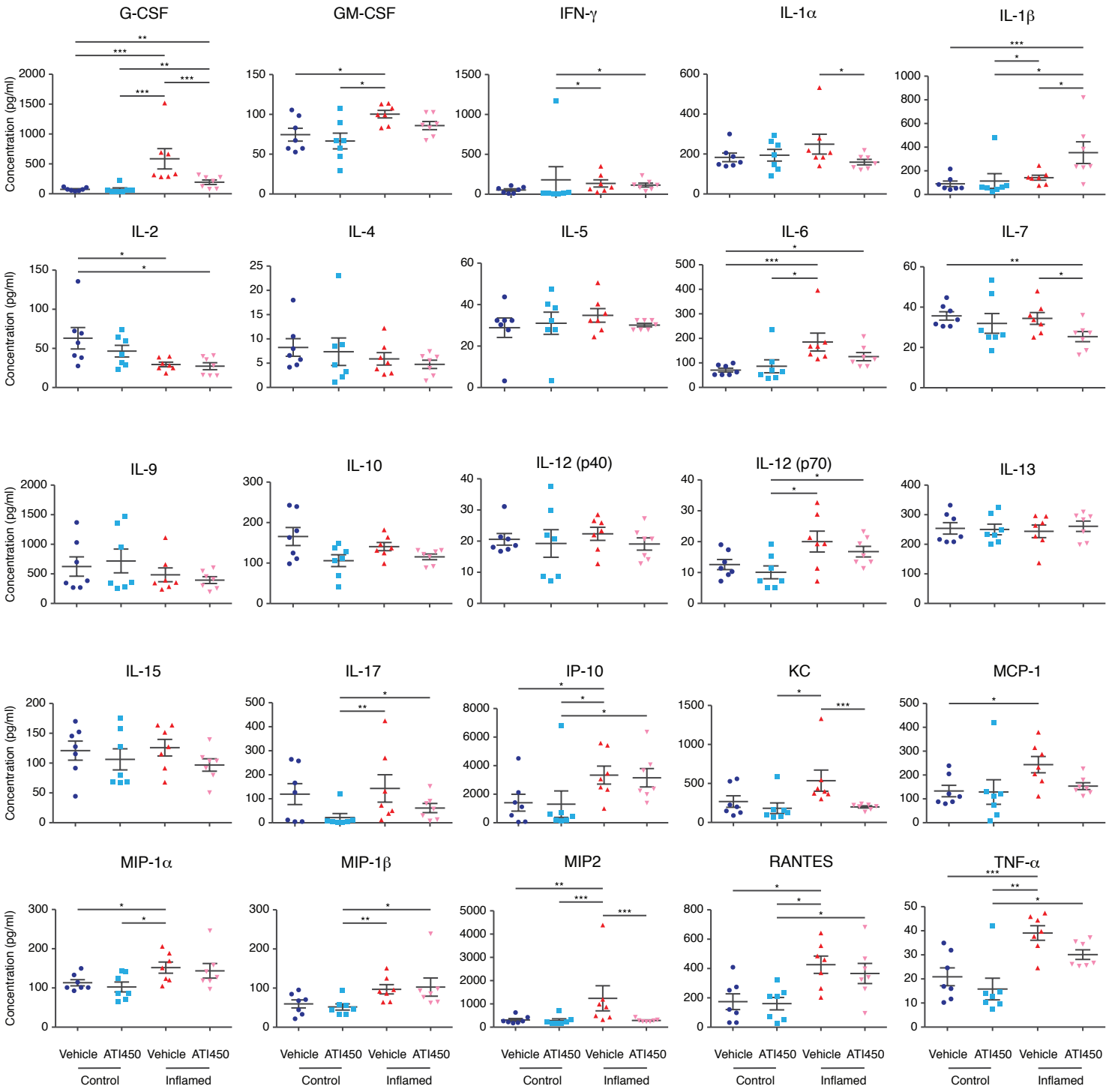

Figure S6. ATI450 reduces the abundance of select cytokines in inflamed colon tissue. Luminex analysis of cytokine abundance in lysates of distal colon tissue from control and inflamed mice with or without ATI450 treatment for 2 weeks, 7 mice/group (3 technical replicates per mouse, averaged). Error bars shown as mean ± SEM, *P< 0.05, **P<0.01, and ***P< 0.005 by Wilcoxon Rank Sum test.

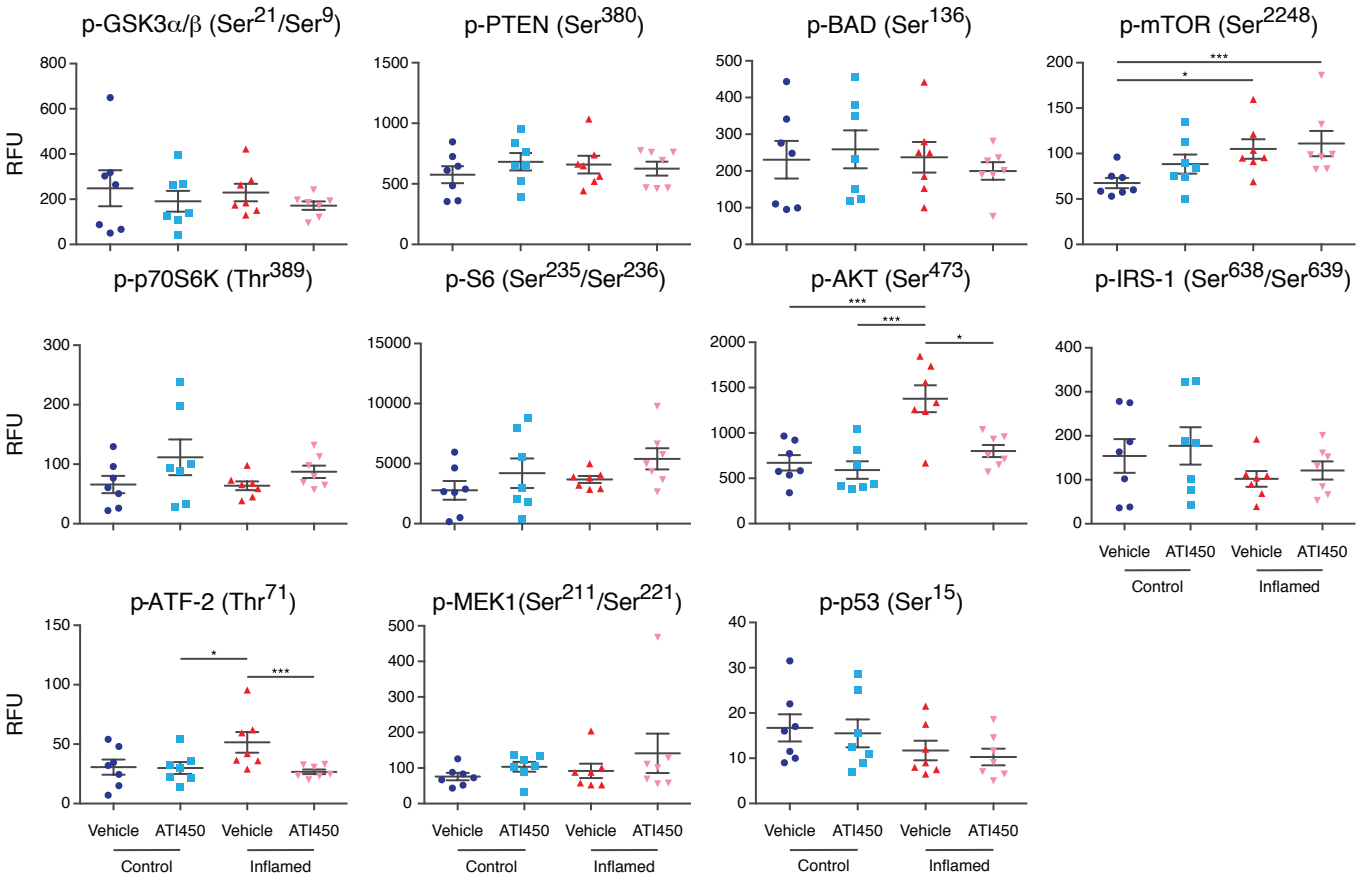

Figure S7. ATI450 reduces the abundance of select phosphoproteins in inflamed colon tissue. Luminex analysis of indicated phosphoproteins in lysates of distal colon tissue from control and inflamed mice with or without ATI450 treatment for 2 weeks. Data are raw fluorescence units (RFU) with 7 mice/group (3 technical replicates per mouse, averaged). Error bars shown as mean ± SEM. *P< 0.05, **P<0.01, and ***P< 0.005 by Wilcoxon Rank Sum test.